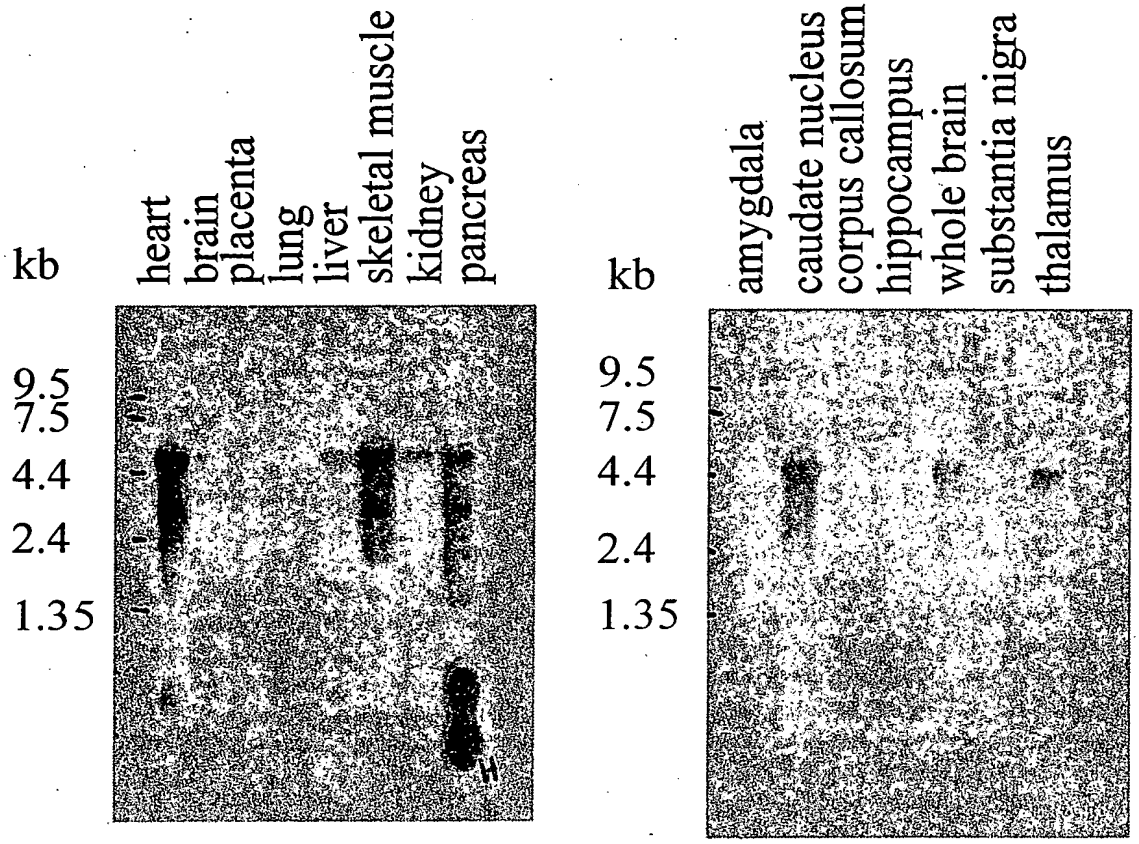


PANEL A

PANEL B

Fig. 1

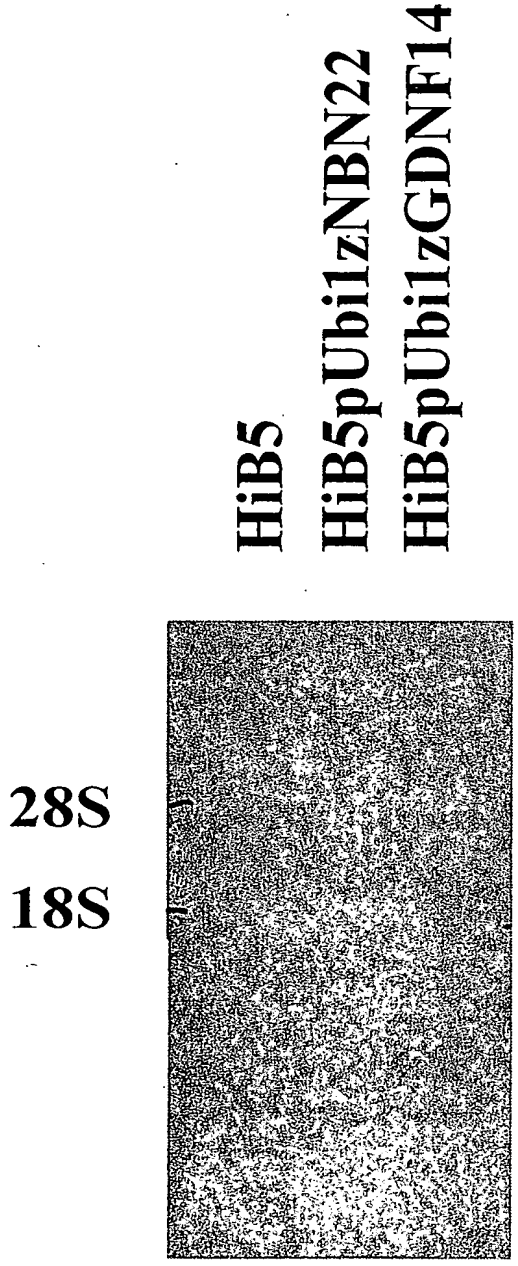


Fig. 2

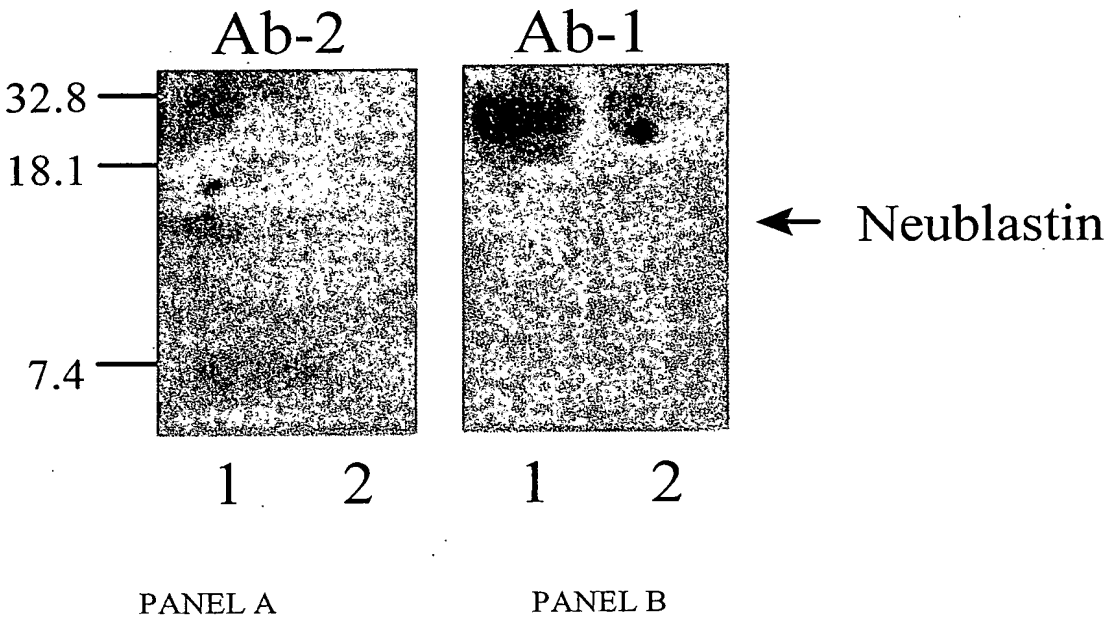
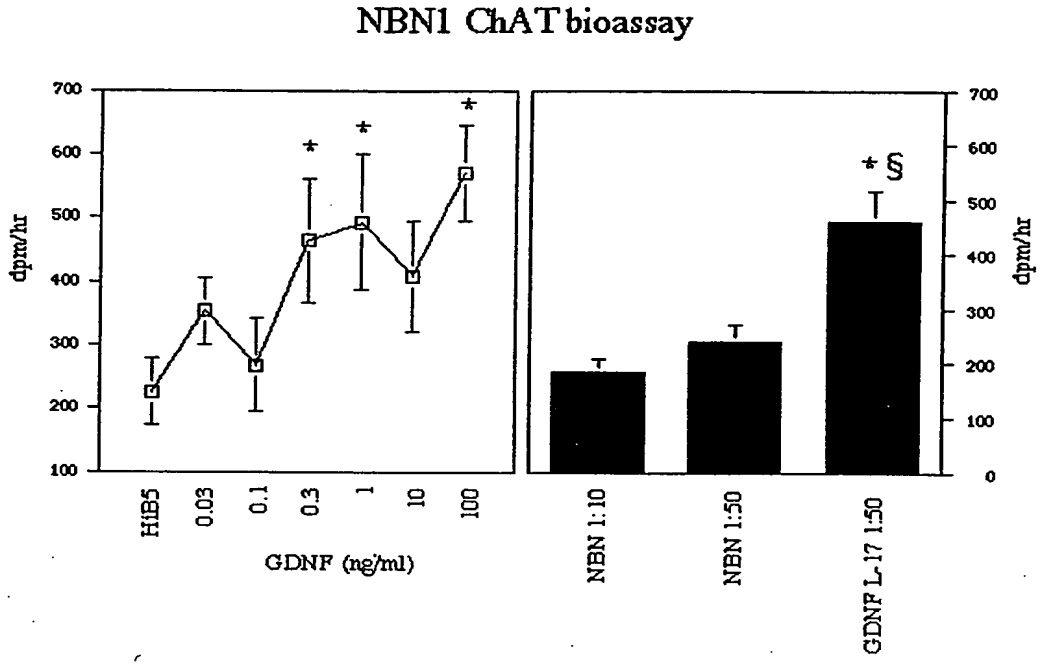
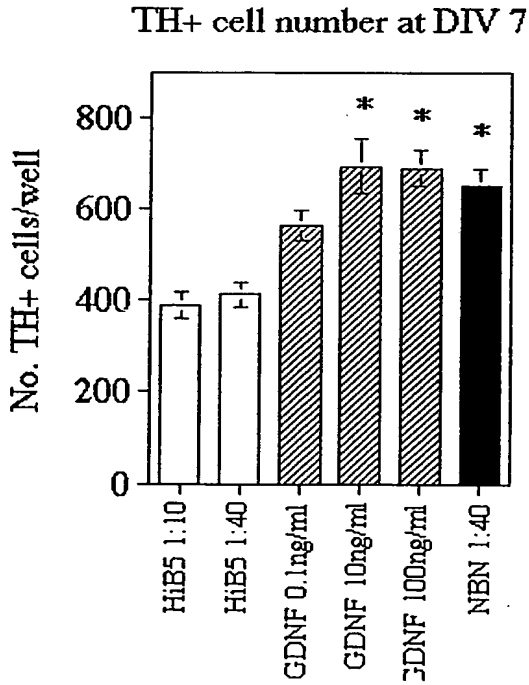


Fig. 3





Figs. 4A, 4B and 4C

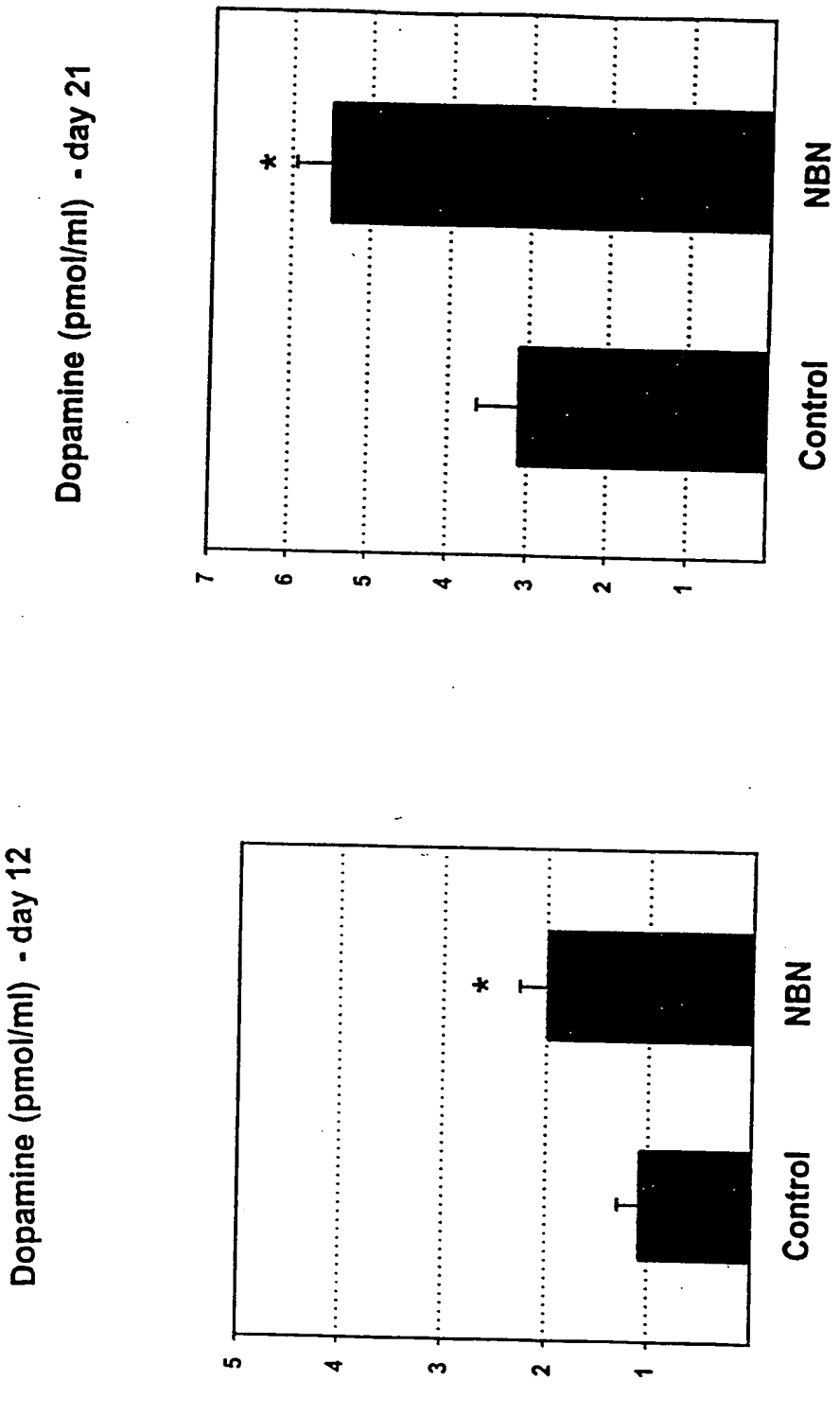

Dopamine (pmol/ml) - day 21
7
6
5
4
3
2
1
*
NBN
Control
Dopamine (pmol/ml) - day 12
5
4
3
2
1
*
NBN
Control

Fig. 5A and 5B

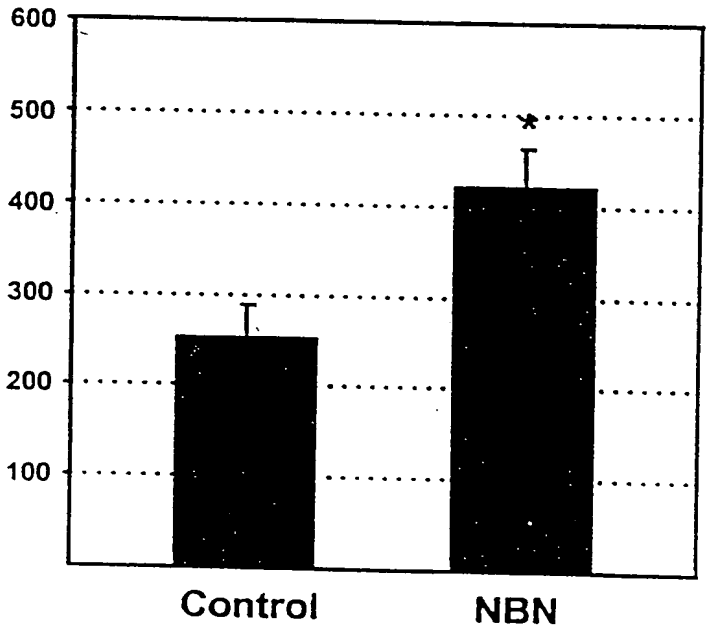


Fig. 5C

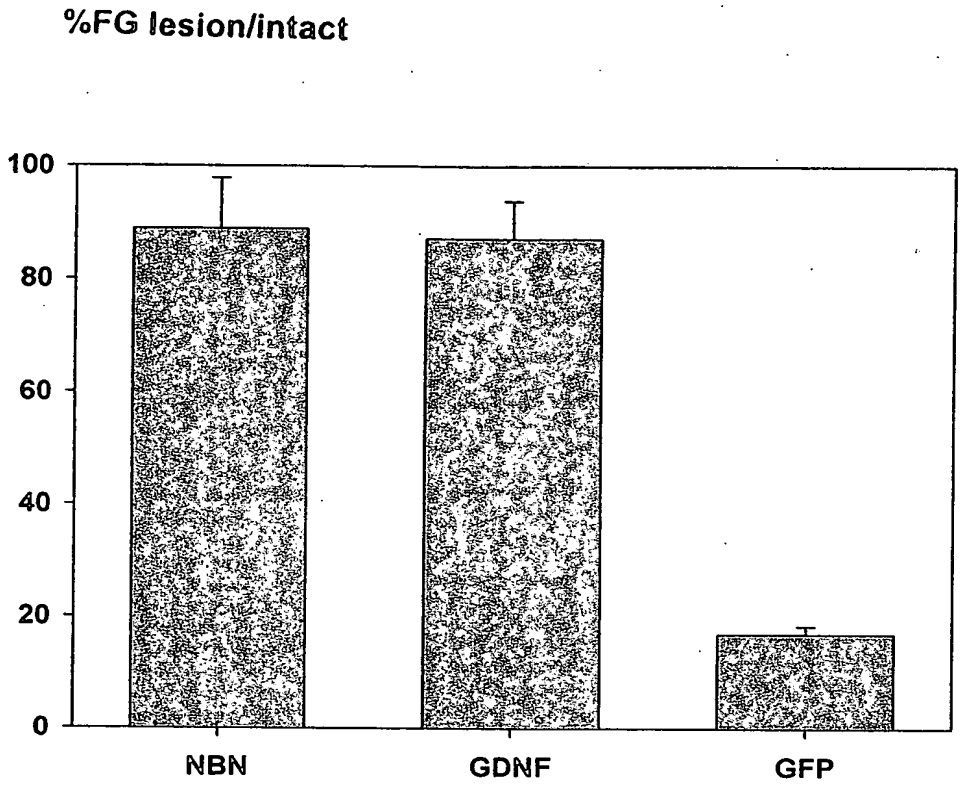


Fig. 6

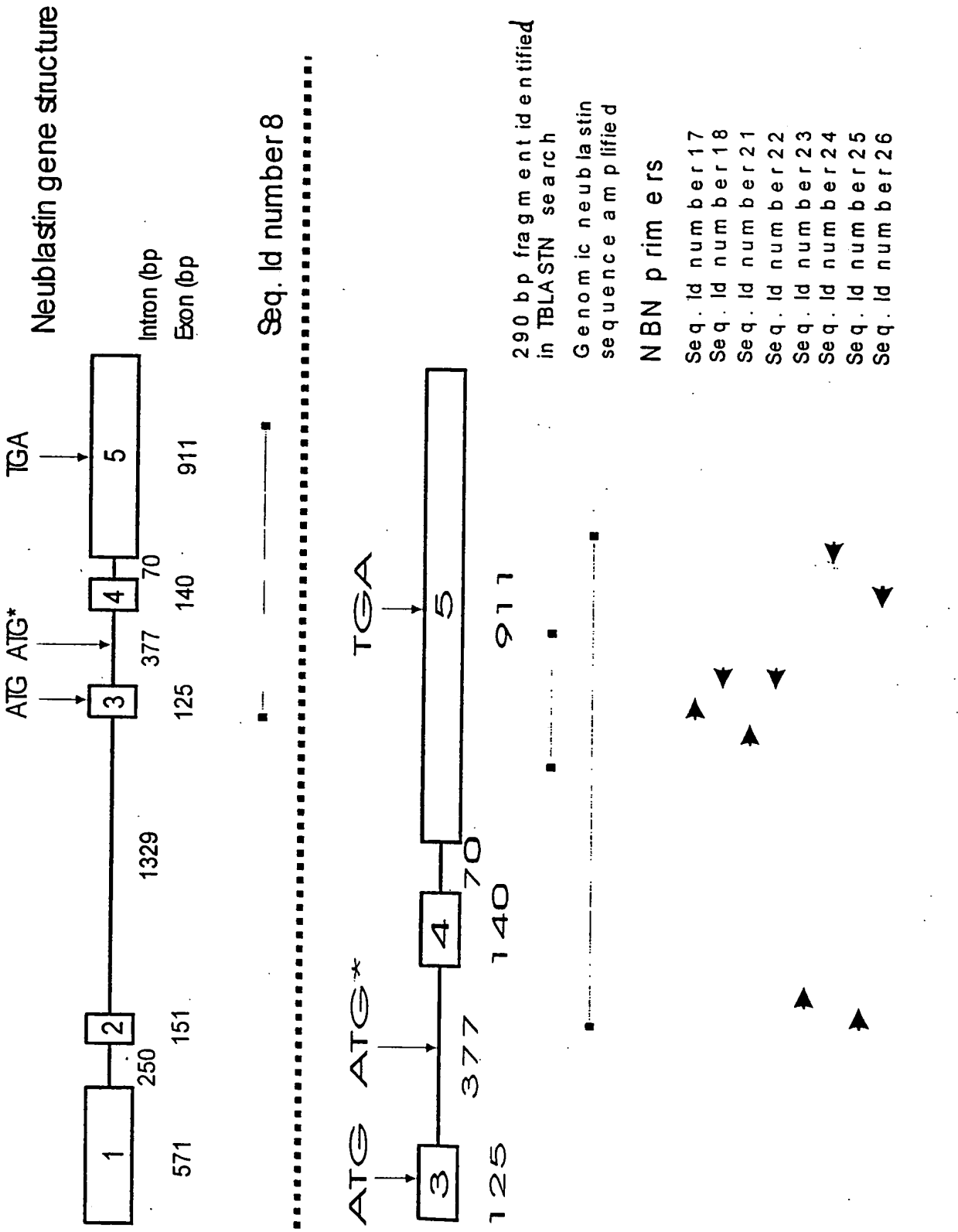

Neublastin gene structure
Intron (bp
Exon (bp
TGA
ATG ATG*
1
2
3
4
5
571
250
151
1329
125
377
140
70
911
Seq. Id number 8
290 bp fragment identified in TBLASTN search
Genomic neublastin sequence amplified
NBN primers
Seq. Id number 17
Seq. Id number 18
Seq. Id number 21
Seq. Id number 22
Seq. Id number 23
Seq. Id number 24
Seq. Id number 25
Seq. Id number 26

Fig. 7

Alignment of Neublastin primers used in Rapid-Screen with homologous regions in other GDNF ligands

5'-**C CTG GCC AGC CTA CTG GG**-3'	**SEQ ID No 17**
G CTG GCC CGG CTG CAG GG	persephin
G CTG CGA CGA **CTG** CGC CA	neurturin
A TTG AAA AAC TTA TCC AG	GDNF

5'-**AA GGA GAC CGC TTC GTA GCG**-3'	**SEQ ID No 18**
TA GGC CAC GTC GGT GTA GCG	persephin
AA GGA CAC CTC GTC CTC GTA GGC	neurturin
AA CGA CAG GTC ATC **ATC AAA** GGC	GDNF

conserved nucleotides shown in **bold**

Fig. 8

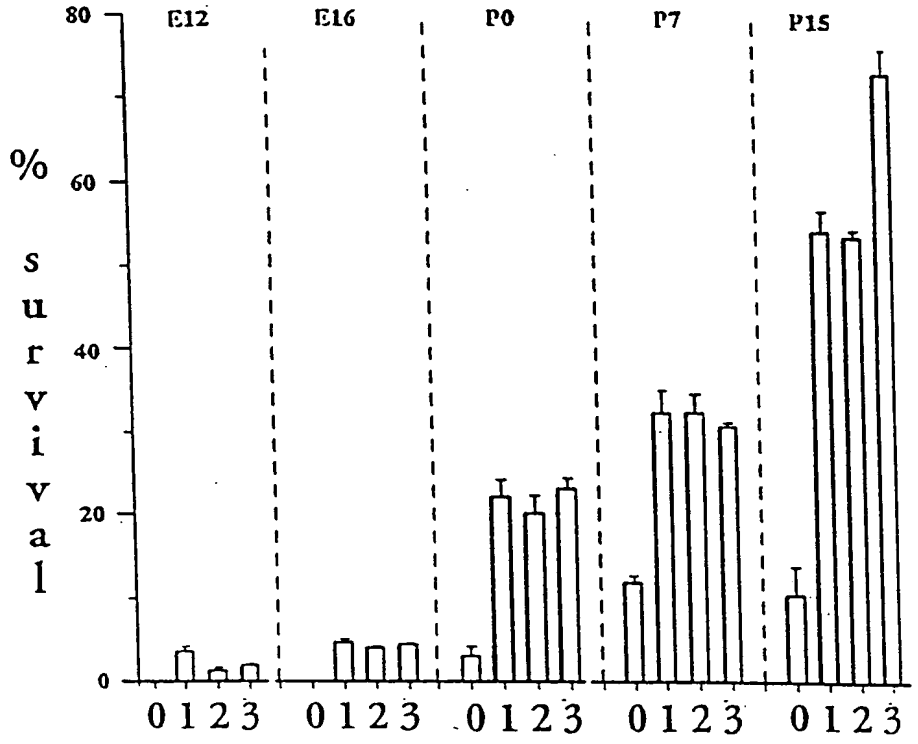

80
60
40
20
0
% survival
E12
E16
P0
P7
P15
0 1 2 3
0 1 2 3
0 1 2 3
0 1 2 3
0 1 2 3

Fig. 9

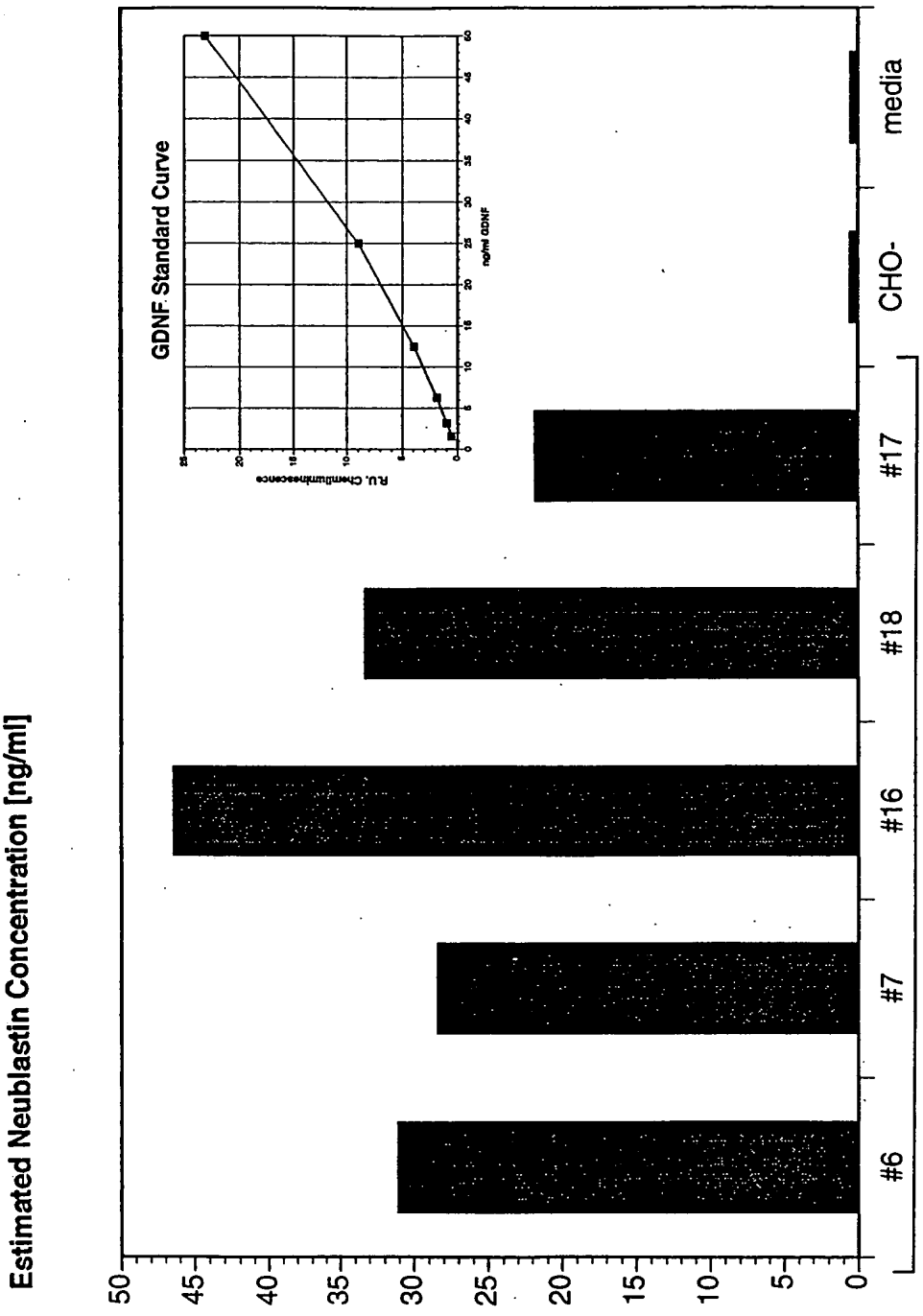

Estimated Neublastin Concentration [ng/ml]
50
45
40
35
30
25
20
15
10
5
0
#6
#7
#16
#18
#17
CHO-
media
CHO Neublastin Clones
GDNF Standard Curve
R.U. Chemiluminescence
ng/ml GDNF

Fig. 10

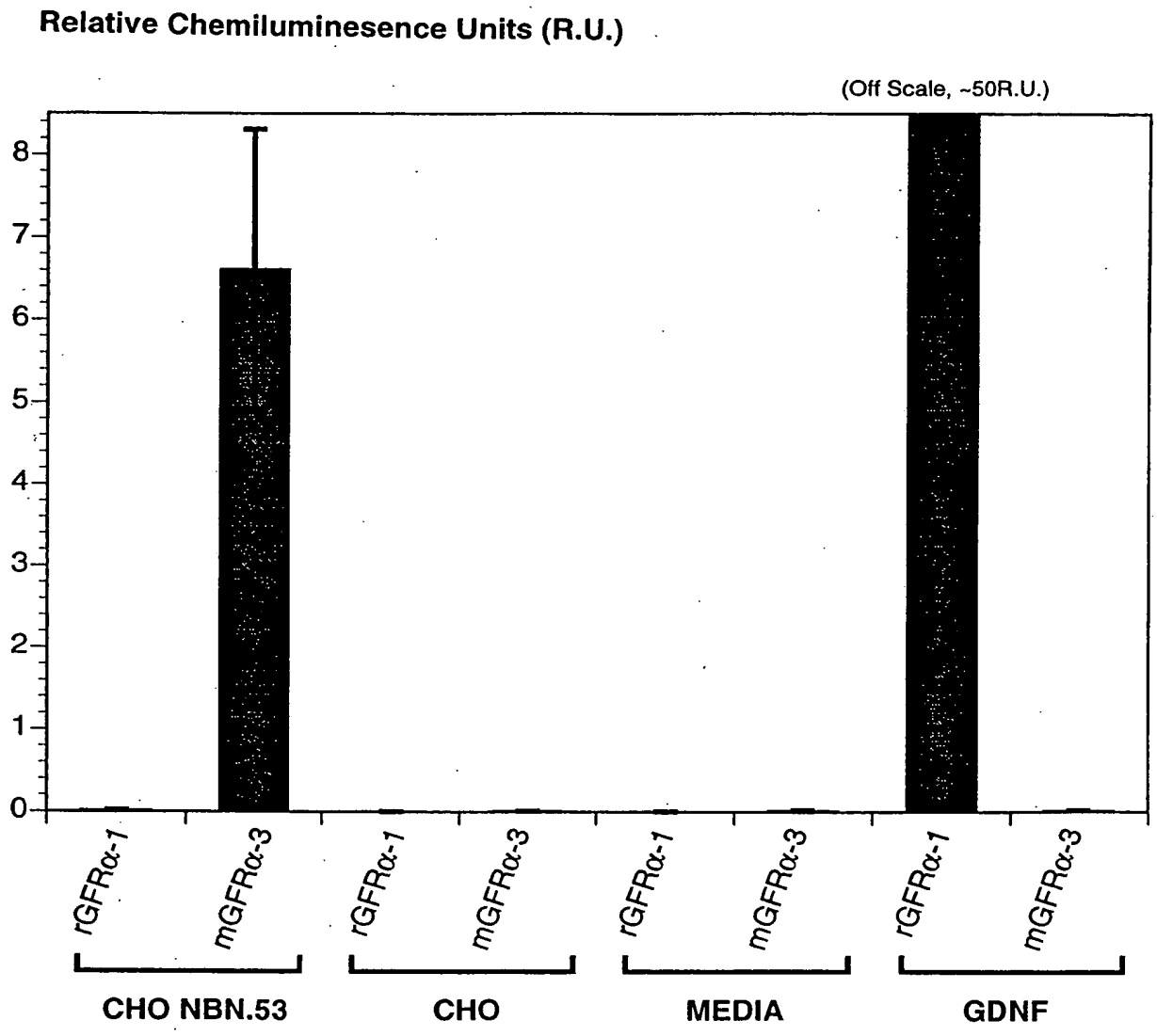
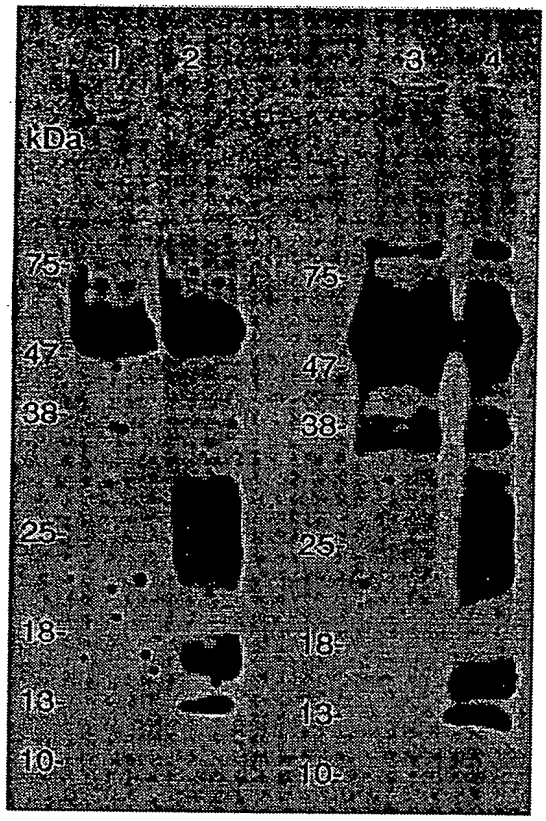
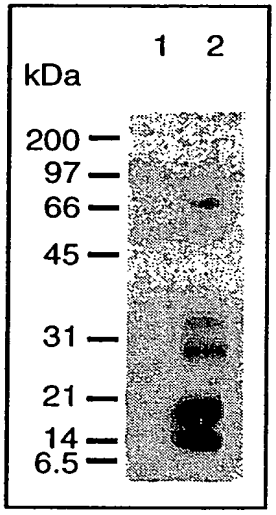

Relative Chemiluminesence Units (R.U.)
(Off Scale, ~50R.U.)
0
1
2
3
4
5
6
7
8
rGFRα-1
mGFRα-3
rGFRα-1
mGFRα-3
rGFRα-1
mGFRα-3
rGFRα-1
mGFRα-3
CHO NBN.53
CHO
MEDIA
GDNF

Fig. 11



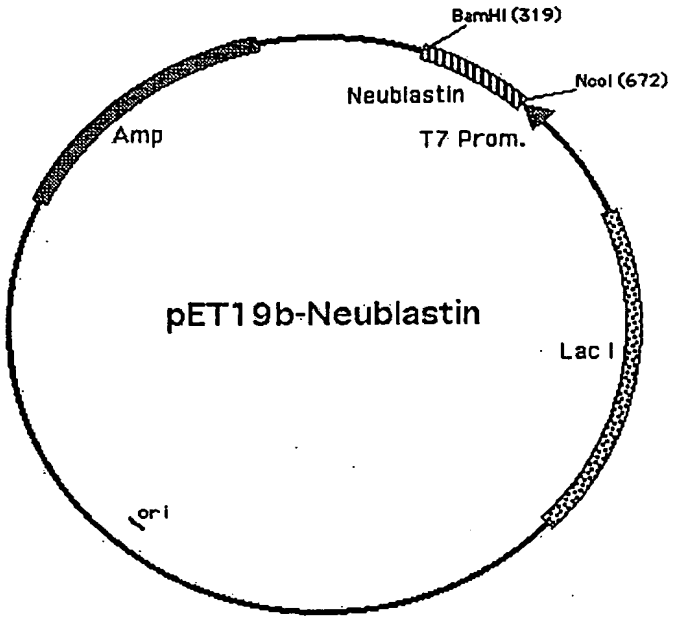
1. Control medium stained with R30 anti-peptide antibody
2. Neublastin containing conditioned medium stained with R30 anti-peptide antibody
3. Control medium stained with R31 anti-peptide antibody
4. Neublastin containing conditioned medium stained with R31 anti-peptide antibody

Fig. 12



Extraction of neublastin by affinity-binding on RETL3-Ig
Lane 1: bound from CHO control conditioned media
Lane 2: bound from neublastin overexpressing CHO conditioned media

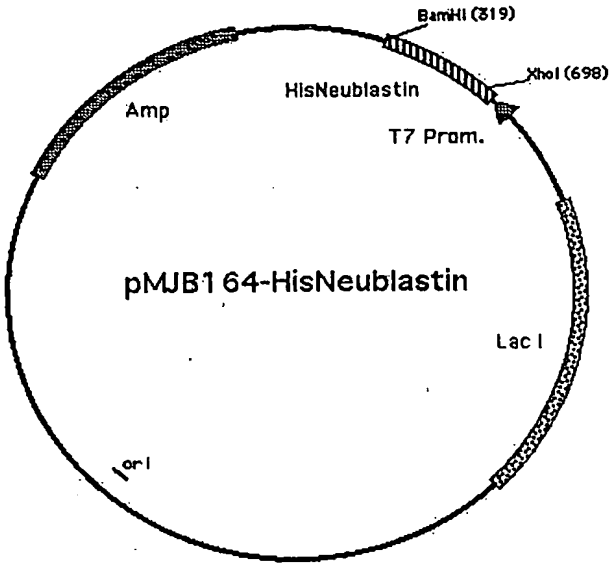
Fig. 13



Neublastin Syngene

```
      NcoI (318)
316   TACCATGGCT GGAGGACCGG GATCTCGTGC TCGTGCAGCA GGAGCACGTG GCTGTCGTCT
      ATGGTACCGA CCTCCTGGCC CTAGAGCACG AGCACGTCGT CCTCGTGCAC CGACAGCAGA
        1▶ M  A    G  G  P    G  S  R  A    R  A  A    G  A  R    G  C  R  L

376   GCGTTCTCAA CTAGTGCCGG TGCGTGCACT CGGACTGGGA CACCGTTCCG ACGAACTAGT
      CGCAAGAGTT GATCACGGCC ACGCACGTGA GCCTGACCCT GTGGCAAGGC TGCTTGATCA
       19▶ R  S  Q    L  V  P    V  R  A  L    G  L  G    H  R  S    D  E  L  V

436   ACGTTTTCGT TTTTGTTCAG GATCTTGTCG TCGTGCACGT TCTCCGCATG ATCTATCTCT
      TGCAAAAGCA AAAACAAGTC CTAGAACAGC AGCACGTGCA AGAGGCGTAC TAGATAGAGA
       39▶ R  F  R    F  C  S    G  S  C  R    R  A  R    S  P  H    D  L  S  L

496   AGCATCTCTA CTAGGAGCCG GAGCACTAAG ACCGCCGCCG GGATCTAGAC CTGTATCTCA
      TCGTAGAGAT GATCCTCGGC CTCGTGATTC TGGCGGCGGC CCTAGATCTG GACATAGAGT
       59▶ A  S  L    L  G  A    G  A  L  R    P  P  P    G  S  R    P  V  S  Q

556   ACCTTGTTGT AGACCTACTA GATACGAAGC AGTATCTTTC ATGGACGTAA ACTCTACATG
      TGGAACAACA TCTGGATGAT CTATGCTTCG TCATAGAAAG TACCTGCATT TGAGATGTAC
       79▶ P  C  C    R  P  T    R  Y  E  A    V  S  F    M  D  V    N  S  T  W

                                                                  BamHI (671)
616   GAGAACCGTA GATAGACTAT CTGCAACCGC ATGTGGCTGT CTAGGATGAT AATAGGGATC
      CTCTTGGCAT CTATCTGATA GACGTTGGCG TACACCGACA GATCCTACTA TTATCCCTAG
       99▶ R  T  V    D  R  L    S  A  T  A    C  G  C    L  G  •    •  •

676   CGGCT
      GCCGA
```

Fig. 14



HisNeublastin

```
                                                    XhoI (340)
301  TACCATGGGC CATCATCATC ATCATCATCA TCATCATCAC TCGAGCGGCC ATATCGACGA
     ATGGTACCCG GTAGTAGTAG TAGTAGTAGT AGTAGTAGTG AGCTCGCCGG TATAGCTGCT
       1 M  G  H  H  H  H  H  H  H  H  H  S  S  G  H  I  D  D

361  CGACGACAAG GCTGGAGGAC CGGGATCTCG TGCTCGTGCA GCAGGAGCAC GTGGCTGTCG
     GCTGCTGTTC CGACCTCCTG GCCCTAGAGC ACGAGCACGT CGTCCTCGTG CACCGACAGC
      19 D  D  K  A  G  G  P  G  S  R  A  R  A  A  G  A  R  G  C  R

421  TCTGCGTTCT CAACTAGTGC CGGTGCGTGC ACTCGGACTG GGACACCGTT CCGACGAACT
     AGACGCAAGA GTTGATCACG GCCACGCACG TGAGCCTGAC CCTGTGGCAA GGCTGCTTGA
      39 L  R  S  Q  L  V  P  V  R  A  L  G  L  G  H  R  S  D  E  L

481  AGTACGTTTT CGTTTTTGTT CAGGATCTTG TCGTCGTGCA CGTTCTCCGC ATGATCTATC
     TCATGCAAAA GCAAAAACAA GTCCTAGAAC AGCAGCACGT GCAAGAGGCG TACTAGATAG
      59 V  R  F  R  F  C  S  G  S  C  R  R  A  R  S  P  H  D  L  S

541  TCTAGCATCT CTACTAGGAG CCGGAGCACT AAGACCGCCG CCGGGATCTA GACCTGTATC
     AGATCGTAGA GATGATCCTC GGCCTCGTGA TTCTGGCGGC GGCCCTAGAT CTGGACATAG
      79 L  A  S  L  L  G  A  G  A  L  R  P  P  P  G  S  R  P  V  S

601  TCAACCTTGT TGTAGACCTA CTAGATACGA AGCAGTATCT TTCATGGACG TAAACTCTAC
     AGTTGGAACA ACATCTGGAT GATCTATGCT TCGTCATAGA AAGTACCTGC ATTTGAGATG
      99 Q  P  C  C  R  P  T  R  Y  E  A  V  S  F  M  D  V  N  S  T

                                                    BamHI (719)
661  ATGGAGAACC GTAGATAGAC TATCTGCAAC CGCATGTGGC TGTCTAGGAT GATAATAGGG
     TACCTCTTGG CATCTATCTG ATAGACGTTG GCGTACACCG ACAGATCCTA CTATTATCCC
     119 W  R  T  V  D  R  L  S  A  T  A  C  G  C  L  G  •  •

721  ATCCGGCTGC TAACAAAGCC CG
     TAGGCCGACG ATTGTTTCGG GC
```

Fig. 15